

PB85-189157

Determination of Neutral Nitrogen-Containing
Pesticides in Industrial and Municipal
Wastewaters - Fenarimol, MGK 264,
MGK 326, and Pronamide

Battelle Columbus Labs., OH

Prepared for

Environmental Monitoring and Support Lab.
Cincinnati, OH

Apr 85



PB85-189157

EPA/600/4-85/018
April 1985

DETERMINATION OF NEUTRAL NITROGEN-CONTAINING
PESTICIDES IN INDUSTRIAL AND MUNICIPAL
WASTEWATERS - FENARIMOL, MGK 264, MGK 326, AND PRONAMIDE

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CINCINNATI, OHIO 45268

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/4-85/018	2.	3. RECIPIENT'S ACCESSION NO. PB8 5 189157 /AS
4. TITLE AND SUBTITLE Determination of Neutral Nitrogen-Containing Pesticides in Industrial and Municipal Wastewaters - Fenarimol, MGK 264, MGK 326, and Pronamide	5. REPORT DATE April 1985	6. PERFORMING ORGANIZATION CODE
	8. PERFORMING ORGANIZATION REPORT NO.	
7. AUTHOR(S) J.S. Warner, T.M. Engel, and P. J. Mondron	10. PROGRAM ELEMENT NO. CBERIC	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Battelle Columbus Laboratories Columbus, OH 43201	11. CONTRACT/GRANT NO. 68-03 2956	
	13. TYPE OF REPORT AND PERIOD COVERED Final Report 8/81 - 9/83	
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Monitoring and Support Laboratory Office of Research and Development 26 West St. Clair Cincinnati, OH 45268	14. SPONSORING AGENCY CODE EPA 600/06	
	15. SUPPLEMENTARY NOTES	
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17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT Distribute to Public	19. SECURITY CLASS (This Report) Not Classified	21. NO. OF PAGES 60
	20. SECURITY CLASS (This page) Not Classified	22. PRICE

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid wastes.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report is one of a series that investigates the analytical behavior of selected pesticides and suggests a suitable test procedure for their measurement in wastewater. The method was modeled after existing EPA methods being specific yet as simplified as possible.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support
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ABSTRACT

A method was developed for the determination of four neutral nitrogen-containing compounds (fenarimol, MGK 264, MGK 326, and pronamide) in wastewaters. The method development program consisted of a literature review; determination of extraction efficiency for each compound from water into methylene chloride; development of a deactivated Florisil cleanup procedure; and determination of suitable gas chromatographic (GC) analysis conditions.

The final method was applied to wastewaters from manufacturers of fenarimol and pronamide in order to determine precision and accuracy. The wastewater from a manufacturer of fenarimol was spiked with the four compounds at the 20 $\mu\text{g/L}$ level. The wastewater from a manufacturer of pronamide was spiked with the four compounds at the 500 $\mu\text{g/L}$ level. Recoveries for the four compounds were in the 74 to 110 percent range at both concentration levels. Method detection limits (MDLs) for the four compounds in distilled water were in the 2 to 6 $\mu\text{g/L}$ range, but may be higher in wastewaters due to interfering compounds.

This report was submitted in partial fulfillment of Contract No. 68-03-2956 by Battelle Columbus Laboratories under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period from August 1, 1981 to September 30, 1983.

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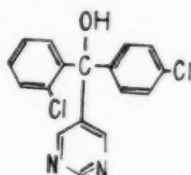
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SECTION 1

INTRODUCTION

Fenarimol, MGK 264, MGK 326, and pronamide are neutral compounds containing carbon, hydrogen, oxygen, nitrogen, and sometimes, chlorine. They were combined into one group (neutral nitrogen containing compounds) for method development purposes only.

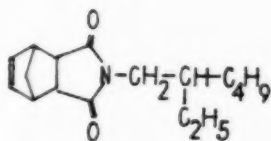
Fenarimol (I) is a fungicide.



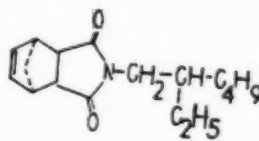
I

Its CAS registry number is 60168-88-9 and its IUPAC name is 2,4'-dichloro-(pyrimidin-5-yl)benzhydroyl alcohol. Other common synonyms include "EL 222" and "Rubigam". The acute oral LD50 of fenarimol in rats is 2500 mg/kg. It is unstable in sunlight. No additional information was found during the literature search.

MGK 264 is used as an insecticidal synergist for pyrethroids and is present in two isomeric forms (II) (III).



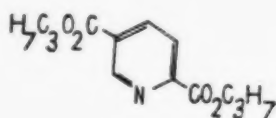
II



III

Its CAS registry number is 136-45-8 and its IUPAC name is N-(2-ethylhexyl)-8,9,10-trinorborn-5-ene-2,3-dicarboximide. Common synonyms include "Octacide 264", "Synergist 264", "Van Dyk 264", and "Sinepyrin 222". Its LD50 in rats is 2800 mg/kg. The literature search yielded descriptions of silica gel and solvent partition cleanup procedures (1), and analyses by gas chromatography-electron capture detector (GC-ECD) using various packed column types (1,2). Infra-red and ultraviolet spectra were reported (3).

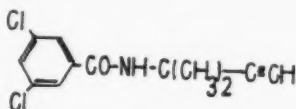
MGK 326 (IV) is a fly repellent.



IV

Its CAS registry number is 113-48-4 and its IUPAC name is dipropyl pyridine-2,5-dicarboxylate. Its other common synonyms include "R-326", "MGK R-326", and "MGK Repellent 326". It is hydrolyzed by alkali and its oral LD50 in rats is 6230 mg/kg. No information on analysis methods for MGK 326 was found from the literature search.

Pronamide (V) is a herbicide used to control grasses in alfalfa, lettuce, and other crops.



V

Its CAS registry number is 23950-58-5 and its IUPAC name is N(1,1-dimethylpropynyl)-3,5-dichlorobenzamide. Some of its synonyms include "Kerb", "Kerb 50W", and "RH 315". The oral LD50 in rats for pronamide is 8350 mg/kg. The literature review revealed an analysis method by which the pronamide is treated with acid to form methyl 3,5-dichlorobenzoate which is subsequently analyzed by GC-ECD (4,5). No direct analysis method for pronamide was found.

The four compounds are neutral, relatively stable in water under normal conditions, and contain nitrogen. For these reasons, the selected approach to the determination of these compounds in water included extraction from water with methylene chloride using separatory funnel techniques, cleanup using Florisil adsorption chromatography and analysis using packed column GC with an alkali flame detector (AFD). The final method is included in Appendix A of this report.

SECTION 2

CONCLUSIONS

EXTRACTION, CONCENTRATION, AND CLEANUP

The four neutral nitrogen-containing pesticides can be extracted into methylene chloride from water and cleaned up on a two percent deactivated Florisil column with greater than 85 percent recovery using standard separatory funnel extraction techniques. Use of Kuderna-Danish (K-D) equipment to perform extract concentrations did not lead to significant recovery losses. The four neutral nitrogen-containing pesticides elute from two percent deactivated Florisil in acetone or less polar solvents. Recoveries greater than 85 percent are obtained. This cleanup procedure was used for wastewaters from manufacturers of fenarimol and pronamide.

CHROMATOGRAPHY

Two packed GC columns, 3% SP-2250 and 3% SP-2100, were found to be acceptable for the GC-AFD analysis of these compounds. The 3% SP-2250 column gave better peak shape for the four compounds and was designated as the primary column. The 3% SP-2100 column was used as the secondary column.

VALIDATION STUDIES

Recoveries of the four neutral nitrogen-containing compounds from water at concentrations ranging from 10 to 1000 $\mu\text{g/L}$ were generally greater than 85 percent resulting in linear analytical curves. The MDLs ranged from 2 to 6 $\mu\text{g/L}$ for all of the compounds. Recoveries of the four compounds from wastewater from a manufacturer of fenarimol at the 20 $\mu\text{g/L}$ level and from wastewater from a manufacturer of pronamide the 500 $\mu\text{g/L}$ level were greater than 85 percent except for MGK 264 at the 500 $\mu\text{g/L}$ level (74 percent recovery).

GC-MS CONFIRMATION

Positive identifications of fenarimol and pronamide in wastewaters were made by GC-MS.

SECTION 3

EXPERIMENTAL

Studies were performed to determine if techniques such as separatory funnel extraction, Florisil adsorption chromatography cleanup, K-D apparatus concentration, and packed column GC-AFD analysis would be applicable to the determination of the four neutral nitrogen-containing compounds in wastewaters. Since recovery data and literature references indicated that these compounds are relatively stable in water, stability studies were not performed.

EXTRACTION, CONCENTRATION, AND CLEANUP

One liter of spiked reagent water was placed in a two-liter separatory funnel and the pH was adjusted to 7 by addition of 6 N sodium hydroxide or 6 N sulfuric acid. The sample was spiked with 100 µg/L of each of the four compounds. The sample was extracted three times with 60 mL each of methylene chloride. The combined extracts were dried by passing them through 10 cm of anhydrous granular sodium sulfate and then concentrated to one mL.

Florisil was activated by heating in a wide-mouth jar at 160-170°C overnight. Florisil, 20 grams, was slurried in 100 mL of ethyl ether containing 400 µl of reagent water. The slurry was transferred to a chromatographic column. The solvent was allowed to elute from the column. Petroleum ether, 25 mL, was added to the column and also allowed to elute from the column. An additional 50 mL of petroleum ether was added to the column. The above mentioned extract was added to the petroleum ether. This solvent was eluted from the column and collected (F1). Nine additional 50 mL solvent elutions were collected: six percent ethyl ether in petroleum ether (F2); 15 percent ethyl ether in petroleum ether (F3); 50 percent ethyl ether in petroleum ether (F4); ethyl ether (F5); six percent acetone in ethyl ether (F6); 15 percent acetone in ethyl ether (F7); 50 percent acetone in ethyl ether (F8); acetone (F9); and six percent methanol in acetone (F10). Each fraction was solvent exchanged to acetone and concentrated to 1 mL.

CHROMATOGRAPHY

Two columns, 3% SP-2250 on 100/120 mesh Supelcoport and 3% SP-2100 on 100/120 mesh Supelcoport, were evaluated for the determination of the four compounds by GC-AFD.

VALIDATION STUDIES

The MDLs for the four compounds were determined by analyzing seven replicate distilled water samples spiked at the 5 µg/L concentration level.(6) The sample extracts were cleaned up using the Florisil cleanup procedure prior to analysis. The amounts recovered were determined by external standard calibration and the MDLs were calculated from these data.

Distilled water was also spiked in duplicate at the 10, 50, 100, 500, and 1000 µg/L concentration levels and recoveries of the four compounds were determined as described earlier. An analytical curve was generated by plotting the amount spiked into the samples versus the amount recovered from the samples.

A wastewater from a manufacturer of fenarimol and a wastewater from a manufacturer of pronamide were used for method validation studies. Seven aliquots of each wastewater were analyzed to determine levels of interferences. The fenarimol wastewater was spiked with the four compounds at the 20 µg/L level, processed and analyzed. The pronamide wastewater was spiked at the 500 µg/L level and also processed and analyzed. Seven replicate extractions were performed at each concentration level.

GC-MS CONFIRMATION

Unspiked extracts from the fenarimol and pronamide wastewaters and stock solutions of fenarimol and pronamide were analyzed by capillary column GC-MS (electron impact (EI) mode) to confirm tentative identifications made of those two compounds.

SECTION 4

RESULTS AND DISCUSSION

EXTRACTION, CONCENTRATION, AND CLEANUP

The four neutral nitrogen-containing pesticides were extracted from distilled water into methylene chloride and cleaned up on two percent deactivated Florisil with greater than 85 percent recovery. Recovery data from duplicate extractions at the 100 µg/L concentration level are given in Table 1.

TABLE 1. EXTRACTION OF NEUTRAL NITROGEN-CONTAINING COMPOUNDS

Compound	Recovery, % (a)
Fenarimol	93, 102
MGK 264(b)	89, 103
MGK 326	90, 107
Pronamide	89, 105

(a) Results from duplicate analyses.

(b) Sum of two isomer peaks.

The four compounds eluted in Florisil fractions 5-9 (ethyl ether, and six percent, 15 percent, and 50 percent acetone in ethyl ether, and acetone). Elution order data are given in Table 2.

TABLE 2. ELUTION ORDERS AND RECOVERIES OF NEUTRAL NITROGEN-CONTAINING COMPOUNDS FROM FLORISIL

Compound	Recovery in Specified Fraction, %					Total
	F5	F6	F7	F8	F9	
Fenarimol		80	22			102
MGK 264		110	3			113
MGK 326				30	70	100
Pronamide	122					122

(a) Elution solvents were 50 mL each of the following:

F5 = 100% ethyl ether
 F6 = 6% acetone in ethyl ether
 F7 = 15% acetone in ethyl ether
 F8 = 50% acetone in ethyl ether
 F9 = 100% acetone

CHROMATOGRAPHY

Both the 3% SP-2250 column and the 3% SP-2100 column were satisfactory for the determination of the four neutral nitrogen-containing compounds. The 3% SP-2250 column, however, gave better peak shape and was chosen as the primary column. The following conditions were used for the primary and alternate columns:

Column: 2m x 2.0mm I.D. 3% SP-2250 on 100/120 mesh Supelcoport or 2m x 2.0mm I.D. 3% SP-2100 on 100/120 mesh Supelcoport
 Detector: Alkali flame detector (AFD), 16 bead volts
 Injector Temperature: 250°C
 Detector Temperature: 300°C
 Oven Temperature: 80°C isothermal for 4 min; programmed from 80°C to 300°C at 8°C/min; 300°C isothermal for 4 min.
 Carrier Gas: Helium at 30 cc/min flow rate

Chromatograms obtained under these conditions are shown in Figures 1 and 2.

VALIDATION STUDIES

The MDLs for the neutral nitrogen-containing compounds ranged from 2 to 6 µg/L. Recoveries and MDL data are given in Table 3.

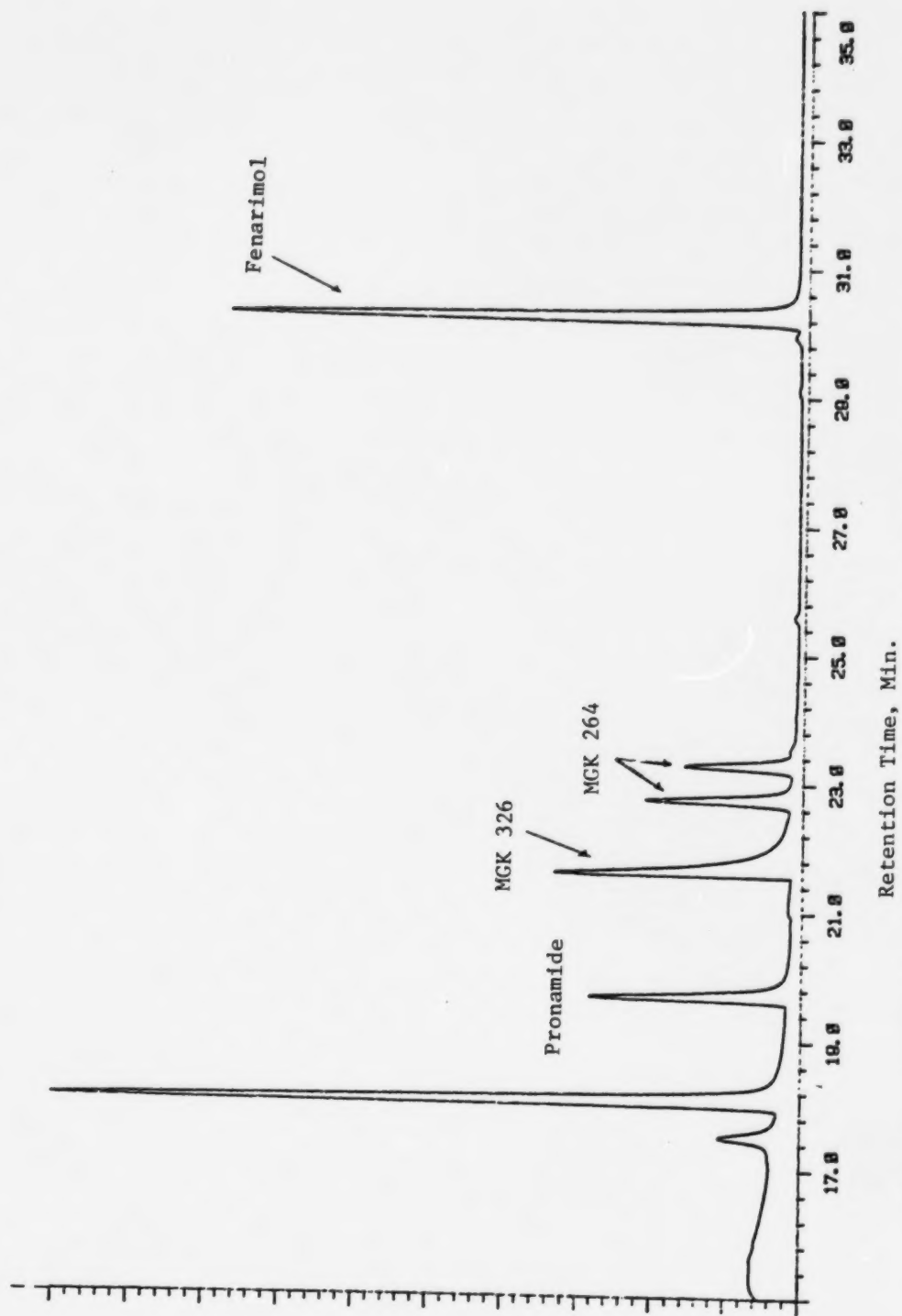


Figure 1. GC-AFD Chromatogram of 100 ng Each of the Neutral Nitrogen-Containing Compounds (Column 1)

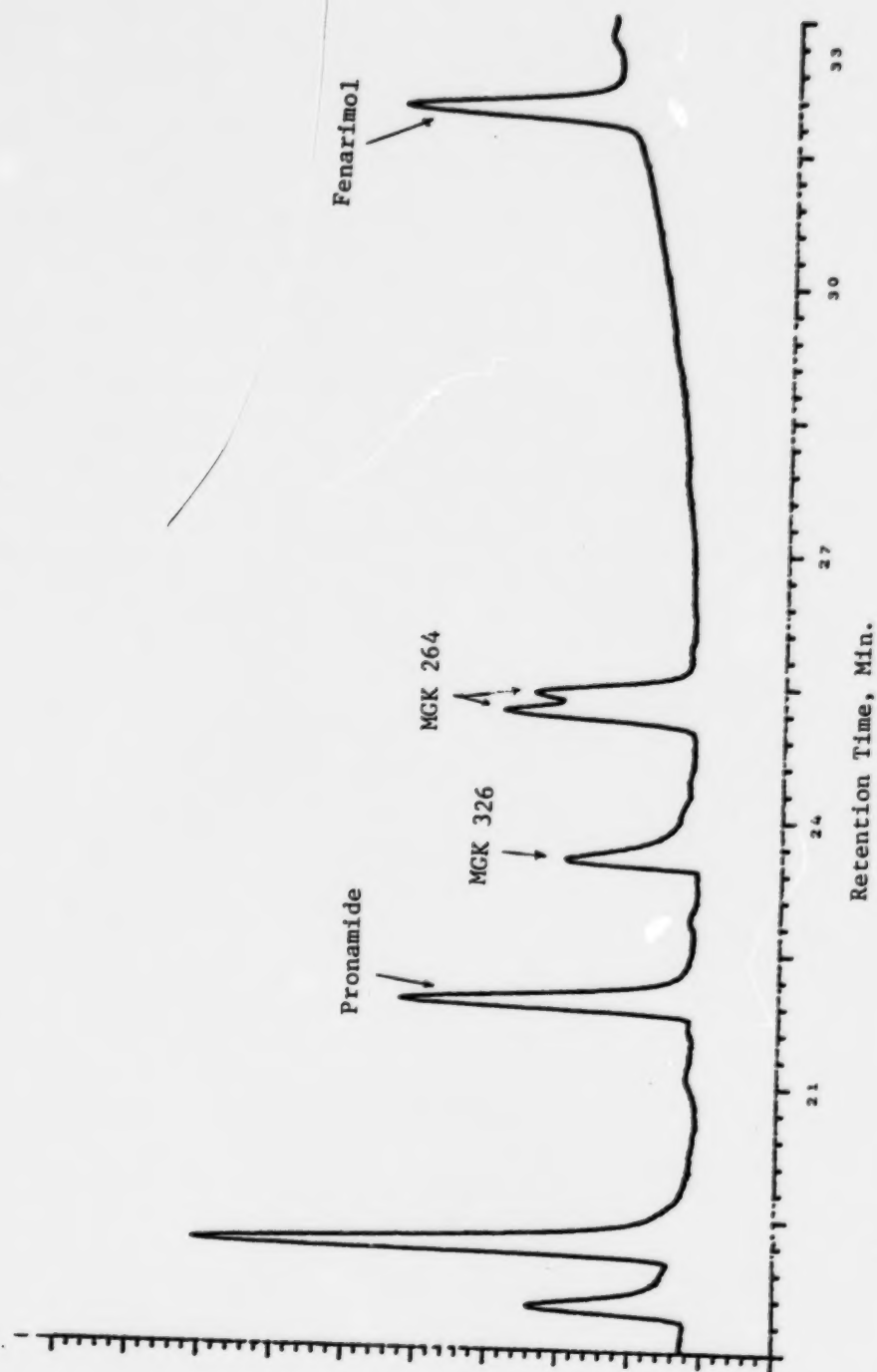


Figure 2. GC-AFD Chromatogram of 200 ng Each of the Neutral Nitrogen-Containing Compounds (Column 2)

TABLE 3. DATA FROM MDL DETERMINATION FOR THE NEUTRAL NITROGEN-CONTAINING COMPOUNDS

Compound	Amount Spiked, µg/L	Amount Recovered, µg/L(a)	MDL, µg/L
Fenarimol	5.0	5.7 ± 0.8	2.4
MGK 264(b)	5.0	4.8 ± 0.6	1.8
MGK 326	5.0	5.6 ± 1.9	6.0
Pronamide	5.0	5.1 ± 1.3	4.1

(a) Average of seven replicate analyses.

(b) Sum of two isomer peaks.

Recoveries of the four compounds obtained during the analytical curve studies were greater than 85 percent except at the 10 µg/ml concentration level for fenarimol, MGK 264, and pronamide. The resultant analytical curves were linear in the concentration range of 10 to 1000 µg/L. Recovery data are given in Table 4. The analytical curves generated from the recovery data are shown in Figures 3-6.

TABLE 4. DATA FROM ANALYTICAL CURVE DETERMINATION FOR THE NEUTRAL NITROGEN-CONTAINING COMPOUNDS

Compound	Amount Recovered at Given Spike Level, µg/L(a)				
	10	50	100	500	1000
Fenarimol	6.6,9.3	41,49	92,100	480,420	990,920
MGK 264(b)	6.6,8.3	49,43	89,100	470,400	1200,870
MGK 326	8.6,9.8	48,43	90,110	500,420	1200,890
Pronamide	5.6,9.2	42,60	89,110	470,410	1100,900

(a) Duplicate analyses.

(b) Sum of two isomer peaks.

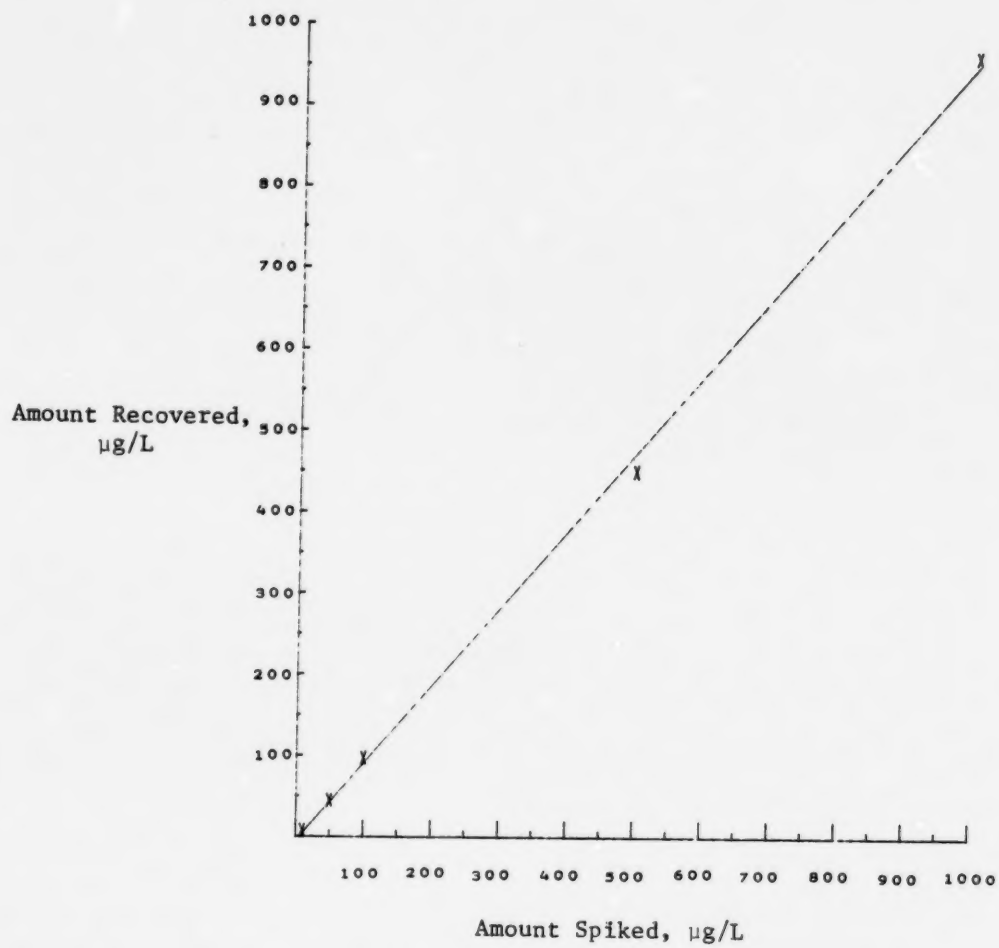


Figure 3. Analytical Curve for Fenarimol

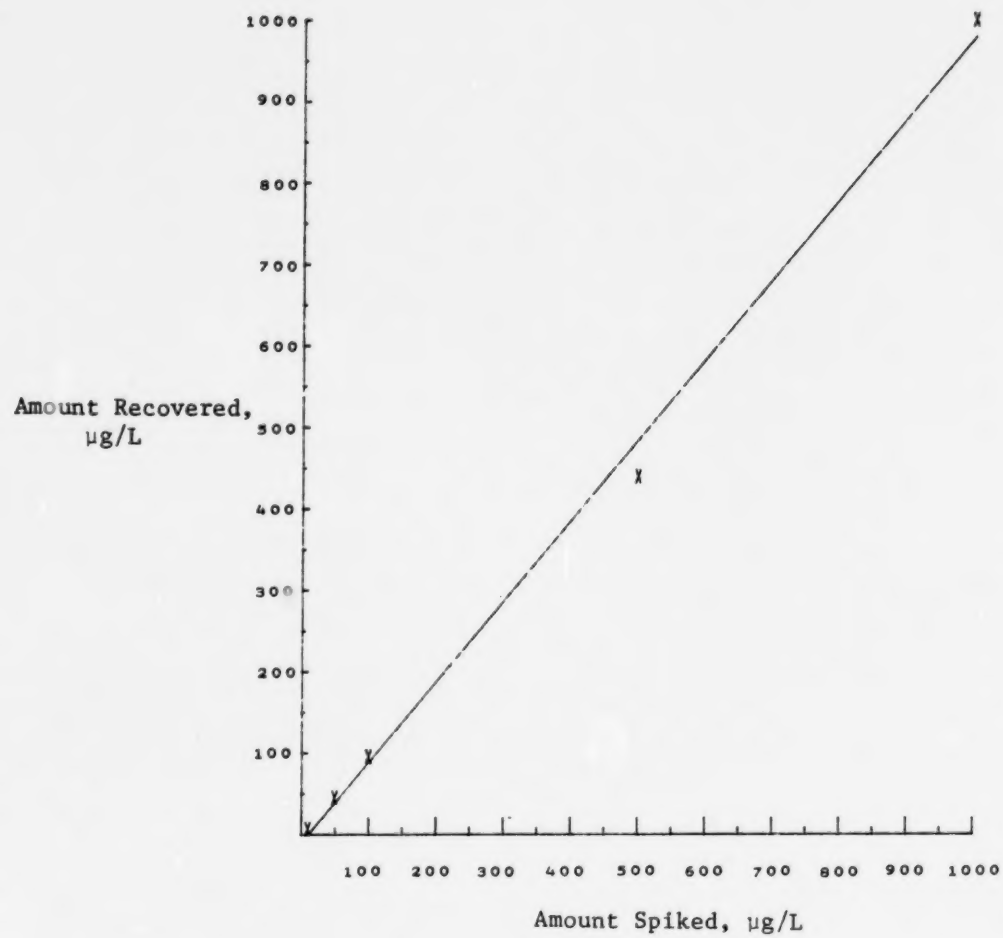


Figure 4. Analytical Curve for MGK 264

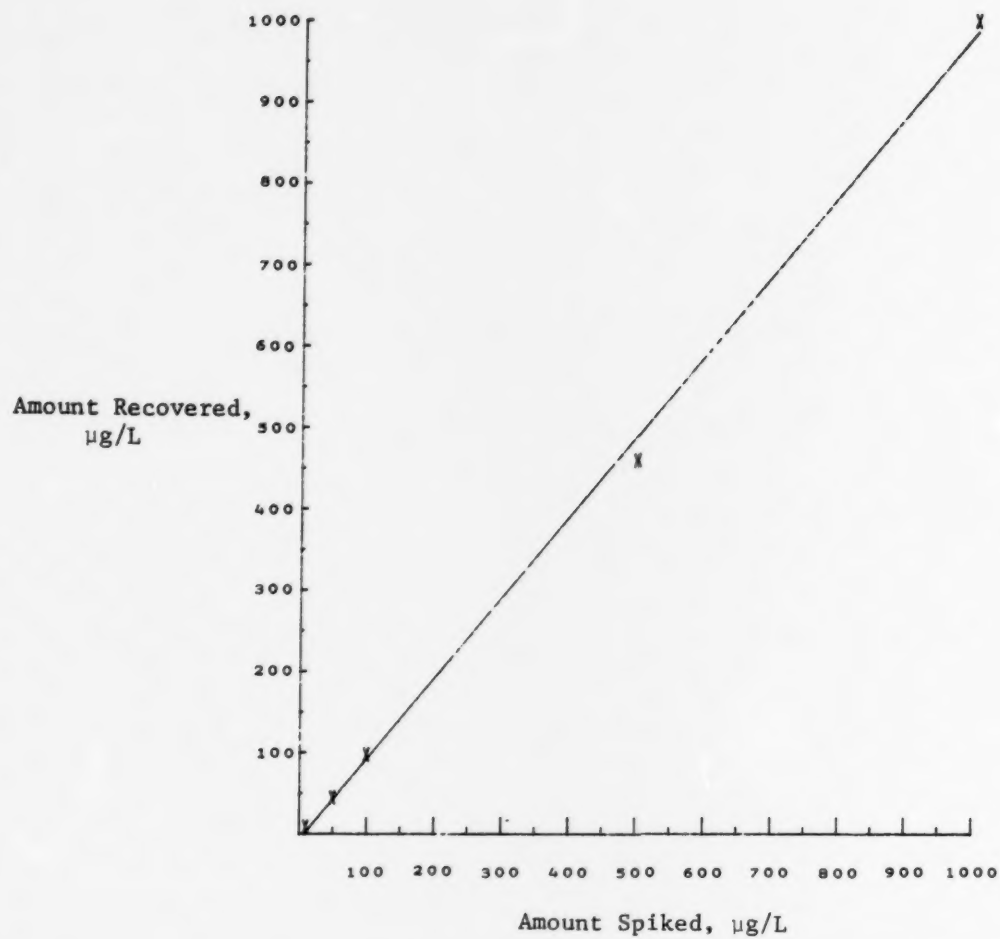


Figure 5. Analytical Curve for MGK 326

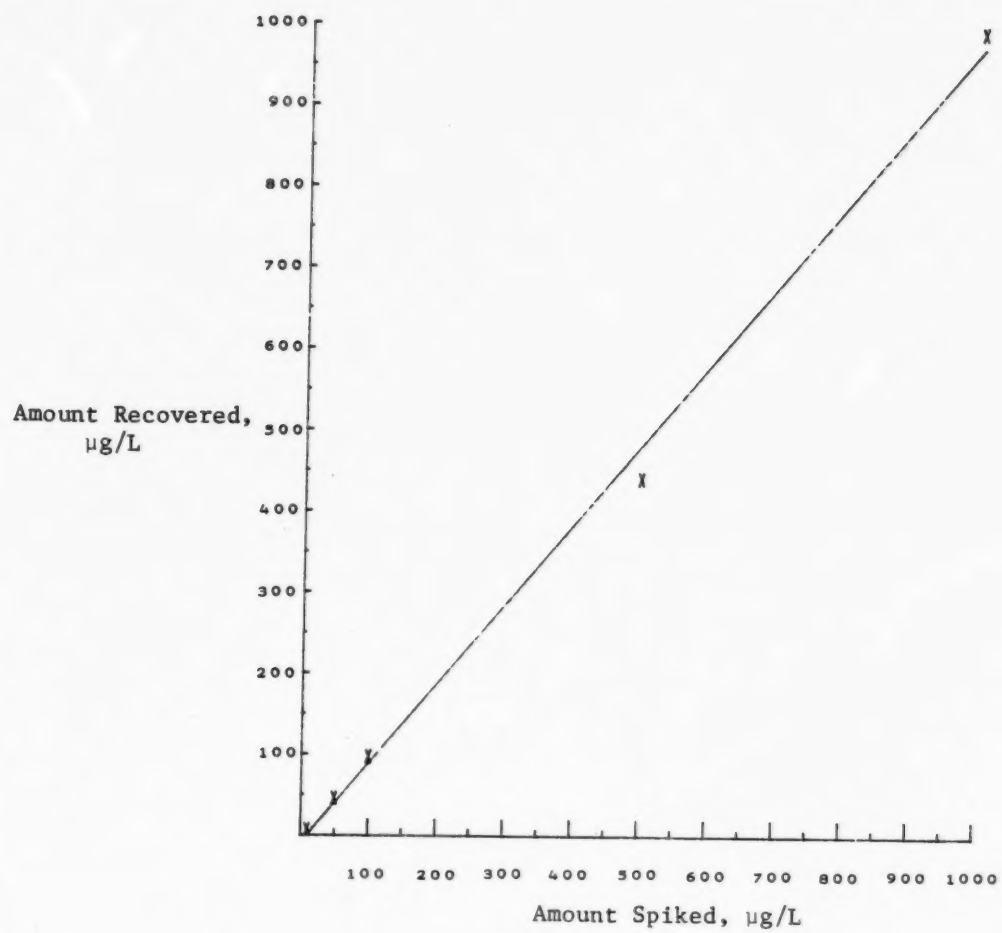


Figure 6. Analytical Curve for Pronamide

Recoveries of the four compounds were greater than 85 percent except for MGK 264 at the 500 $\mu\text{g/L}$ level (74 percent recovery). Seven one-liter aliquots of unspiked fenarimol wastewater were analyzed and fenarimol was tentatively identified at the $1.8 \pm 0.3 \mu\text{g/L}$ level. Similarly, pronamide was tentatively identified in the pronamide wastewater at the $210 \pm 30 \mu\text{g/L}$ level. Recovery data were corrected for the fenarimol and pronamide levels in the wastewaters. Recovery data from low and high level validations in wastewaters are given in Table 5. Chromatograms of spiked and unspiked fenarimol wastewater extracts and spiked and unspiked pronamide wastewaters are given in Figures 7 and 8, respectively.

TABLE 5. RECOVERIES FROM VALIDATION OF NEUTRAL NITROGEN-CONTAINING COMPOUNDS METHOD

Compound	Wastewater(c)	Spike Level, $\mu\text{g/L}$	Recovery, %(a)	Relative Standard Deviation, %
Fenarimol	1	20	98	4.4
	2	500	96	4.4
MGK 264(b)	1	20	96	22
	2	500	74	3.5
MGK 326	1	20	110	6.6
	2	500	95	3.7
Pronamide	1	20	100	4.9
	2	500	86	3.3

(a) Average of seven replicate analyses.

(b) Sum of two isomer peaks

(c) 1 = Wastewater from a manufacturer of fenarimol.
2 = Wastewater from a manufacturer of pronamide.

GC-MS CONFIRMATION

The presence of fenarimol in the fenarimol wastewater and pronamide in the pronamide wastewater were confirmed by GC-MS on the basis of capillary column retention time and mass spectra. Mass spectra of fenarimol and pronamide (in a standard mix and in unspiked wastewater extracts) are given in Figures 9 and 10, respectively.

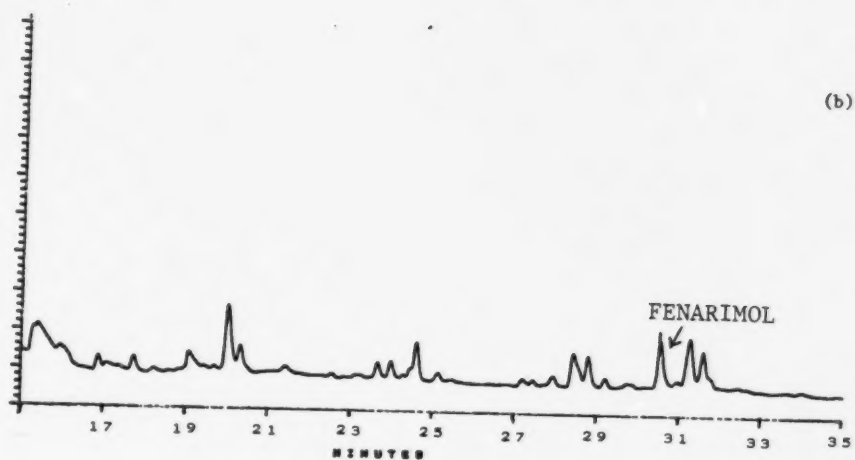
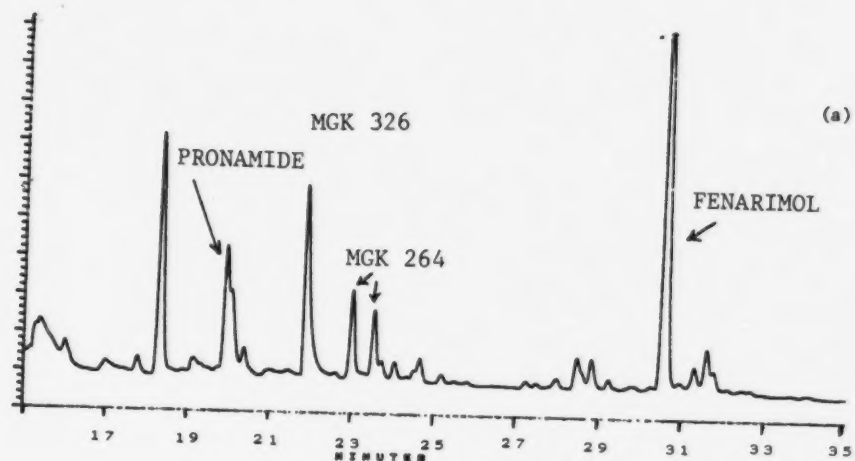


Figure 7. GC-AFD Chromatograms of Fenarimol Wastewater (a) Spiked at the 20 $\mu\text{g/L}$ Level with the Neutral Nitrogen-Containing Compounds and (b) Unspiked.

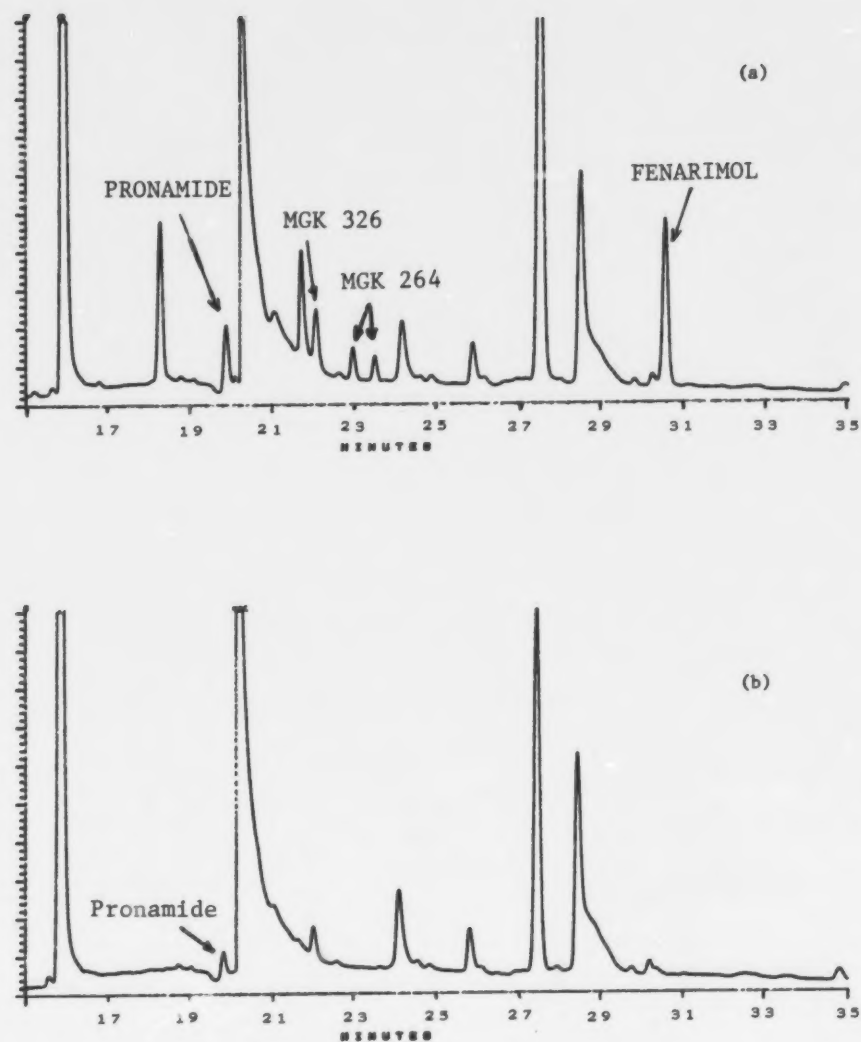


Figure 8. GC-AFD Chromatograms of Pronamide Wastewater (a) Spiked at the 500 µg/L Level with the Neutral Nitrogen-Containing Compounds and (b) Unspiked.

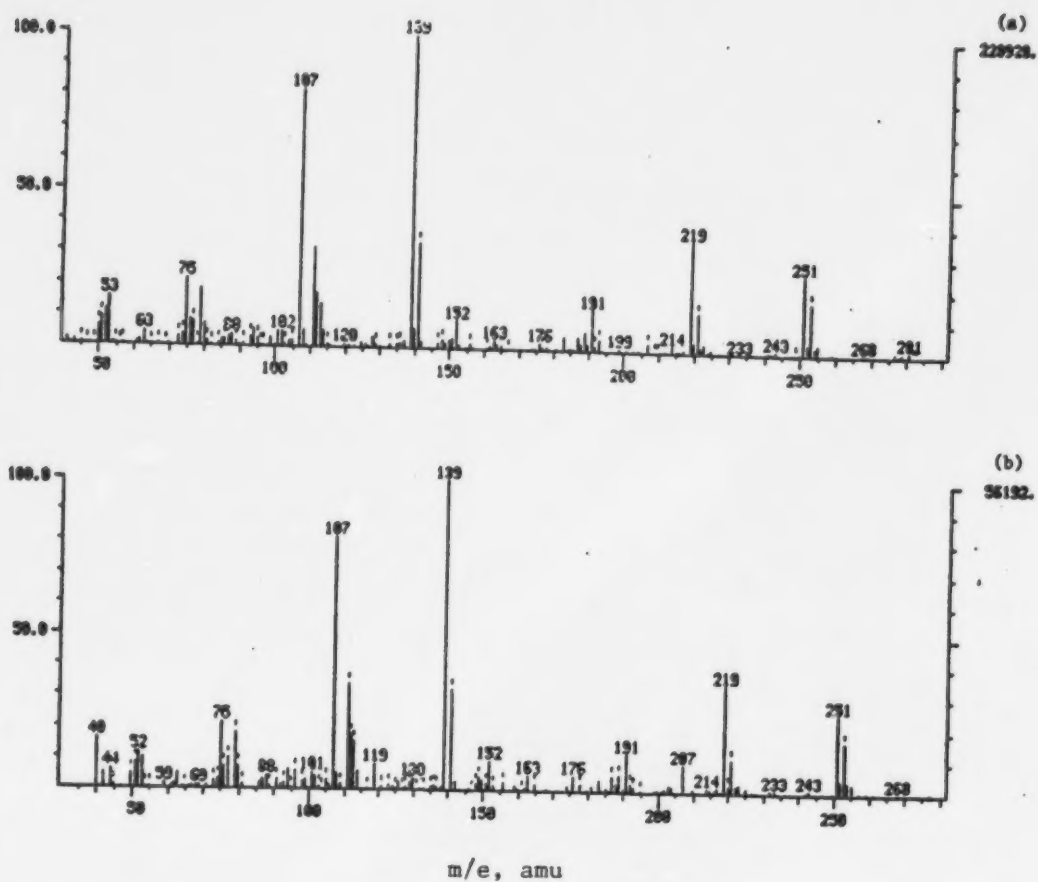


Figure 9. EI Mass Spectra of (a) Fenarimol Standard and (b) Fenarimol Found in Fenarimol Wastewater.

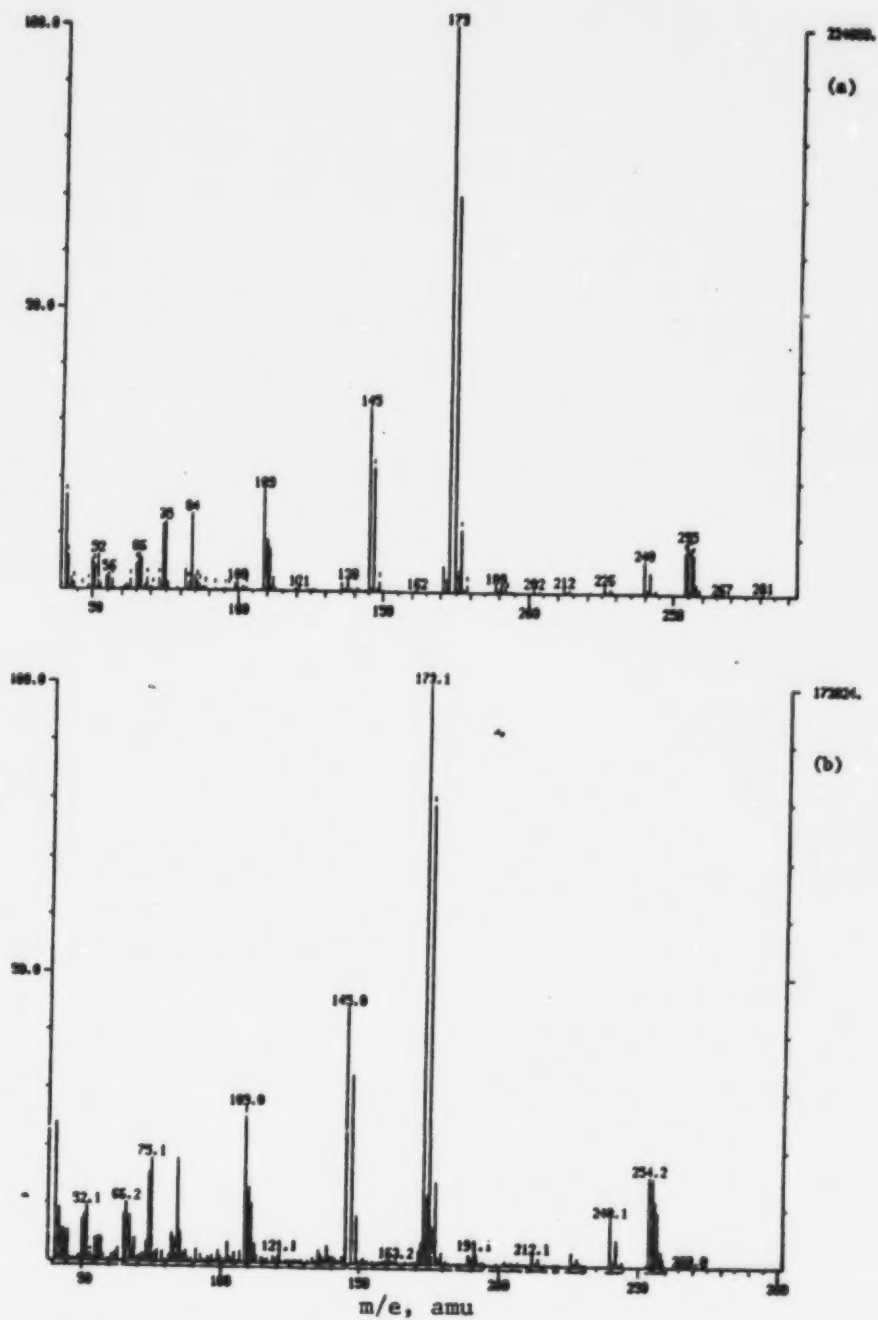


Figure 10. EI Mass Spectra of (a) Pronamide Standard and (b) Pronamide Identified in Pronamide Wastewater

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NEUTRAL NITROGEN-CONTAINING PESTICIDES

METHOD 633.1

1. Scope and Application

- 1.1 This method covers the determination of certain neutral nitrogen-containing pesticides. The following parameters can be determined by this method:

<u>Parameter</u>	<u>CAS No.</u>
Fenarimol	60168-88-9
MGK 264-A	136-45-8
MGK 264-B	136-45-8
MGK 326	113-38-4
Pronamide	23950-58-5

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. Any modification of this method beyond those expressly permitted shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.
- 1.3 The method detection limit (MDL, defined in Section 15) for each compound is listed in Table 2. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.
- 1.4 The sample extraction and concentration steps in this method are similar to those in other 600 series methods. Thus, a single sample may be extracted to measure the compounds included in the

scope of the methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, in order to apply appropriate cleanup procedures.

- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.
- 1.6 When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Section 14 provides gas chromatograph/mass spectrometer (GC-MS) criteria appropriate for the qualitative confirmation of compound identifications.

2. Summary of Method

- 2.1 A measured volume of sample, approximately 1 liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to 1.0 mL. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by alkali flame detector gas chromatography (GC-AFD).¹
- 2.2 This method provides an optional Florisil column cleanup procedure to aid in the elimination of interferences which may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.² Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry, and heat in an oven or muffle furnace at 400°C for 15 to 30 min. Do not heat volumetric ware. Some thermally stable materials such as PCBs may not be eliminated by this treatment. Thorough rinsing with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. After drying and cooling, seal and store glassware in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences

will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 2.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified 3-5 for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber borosilicate or flint glass, 1-liter or 1-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner

must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only.)

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 10 mm ID with coarse frit.

5.2.3 Chromatographic column - 400 mm long x 19 mm ID with 250 mL reservoir at the top and Teflon stopcock (Kontes K-420290 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. A ground glass stopper is used to prevent evaporation of extracts.

- 5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.
- 5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).
- 5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).
- 5.2.8 Vials - Amber glass, 10 to 15 mL capacity with Teflon lined screw-cap.
- 5.2.9 Erlenmeyer flask - 250-mL.
- 5.2.10 Graduated cylinder - 1000-mL.
- 5.2.11 Beaker - 250-mL.
- 5.3 Boiling chips - Approximately 10/40 mesh carborundum. Heat to 400°C for 4 hours or Soxhlet extract with methylene chloride.
- 5.4 Water bath - Heated, capable of temperature control ($\pm 2^{\circ}\text{C}$). The bath should be used in a hood.
- 5.5 Balance - Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.
 - 5.6.1 Column 1 - 180 cm long x 2 mm ID glass, packed with 3% SP-2250 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance

statements in Section 15. Alternate columns may be used in accordance with the provisions described in Section 12.1.

5.6.2 Column 2 - 180 cm long x 2 mm ID glass, packed with 3% SP-2100 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector - Alkali-flame detector (AFD), sometimes referred to as a nitrogen-phosphorous detector (NPD) or a thermionic specific detector (TSD). This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope and was used to develop the method performance statements in Section 15.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Methylene chloride, methanol, petroleum ether, ethyl ether, acetone, distilled-in-glass quality or equivalent. Ethyl ether must be free of peroxides as indicated by EM Quant Test Strips (available from Scientific Products Co., Catalog No. P1126-8 and other suppliers). Procedures recommended for removal of peroxides are provided with the test strips.

6.3 6N Sodium hydroxide - Dissolve 24.0 grams NaOH in 100 mL of reagent water.

6.4 6N Sulfuric acid - Slowly add 16.7 mL of conc. H_2SO_4 (94%) to about 50 mL of reagent water. Dilute to 100 mL with reagent water.

6.5 Sodium sulfate (ACS) Granular, anhydrous; heated in a muffle furnace at 400°C overnight.

6.6 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in brown glass bottle. To prepare for use, place 150 g

in a wide-mouth jar and heat overnight at 160-170°C. Seal tightly with Teflon or aluminum foil-lined screw cap and cool to room temperature.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in distilled-in-glass quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Frequently check stock standard solutions for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

6.7.3 Stock standard solutions must be replaced after 6 months or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 2. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 For each compound of interest, prepare calibration standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates or should define the working range of the detector.

7.2.2 Using injections of 1 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each compound at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, the average calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Due to these limitations, no internal standard applicable to all samples can be suggested.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each compound of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetone. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the range of concentrations expected in the sample concentrates, or should define the working range of the detector.

7.3.2 Using injections of 1 to 5 μL of each calibration standard, tabulate the peak height or area responses against the

concentration for each compound and internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_S C_{IS}) / (A_{IS} C_S)$$

where:

A_S = Response for the compound to be measured.

A_{IS} = Response for the internal standard.

C_{IS} = Concentration of the internal standard in $\mu\text{g/L}$.

C_S = Concentration of the compound to be measured in $\mu\text{g/L}$.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_S/A_{IS} against RF.

7.3.3 The working calibration curve or RF must be verified on each working shift by the measurement of one or more calibration standards. If the response for any compound varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

- 8.1 Each laboratory using this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated.
 - 8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.
 - 8.1.2 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 8.2.
 - 8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.
- 8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.
 - 8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations.

- 8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.
- 8.2.3 Calculate the average percent recovery (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.
- 8.2.4 Using the appropriate data from Table 3, determine the recovery and single operator precision expected for the method, and compare these results to the values measured in Section 8.2.3. If the data are not comparable, the analyst must review potential problem areas and repeat the test.
- 8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.
- 8.3.1 Calculate upper and lower control limits for method performance as follows:
- Upper Control Limit (UCL) = $R + 3s$
- Lower Control Limit (LCL) = $R - 3s$
- where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts⁶ that are useful in observing trends in performance.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.⁶

8.4 The laboratory is required to collect in duplicate a portion of their samples to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular compound does not fall within the control limits for method performance, the results reported for that compound in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of 1-liter aliquot of reagent water that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Samples Collection, Preservation, and Handling

- 9.1 Grab samples must be collected in glass containers. Conventional sampling practices⁷ should be followed; however, the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of plastic and other potential sources of contamination.
- 9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.
- 9.3 Adjust the pH of the sample to 6 to 8 with 6N sodium hydroxide or 6N sulfuric acid immediately after sampling.

10. Sample Extraction

- 10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2-liter separatory funnel. Check the pH of the sample with wide range pH paper and adjust to 6 to 8 with 6 N sodium hydroxide or 6 N sulfuric acid.
- 10.2 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.
- 10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.
- 10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

- 10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer. Once the flask rinse has passed through the drying column, rinse the column with 30 to 40 mL of methylene chloride.
- 10.6 Add 1 or 2 clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the macro Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath, 60 to 65°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 10.7 Remove the macro-Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. Add 1 or 2 clean boiling chips and attach a two-ball micro-Snyder column to the concentrator tube. Prewet the micro-Snyder column with methylene chloride and concentrate the solvent extract as before. When an apparent volume of 0.5 mL is reached, or the solution stops boiling, remove the K-D apparatus and allow it to drain and cool for 10 minutes.

- 10.8 Remove the micro-Snyder column and adjust the volume of the extract to 1.0 mL with methylene chloride. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extract is to be stored longer than 2 days, transfer the extract to a screw-capped vial with a Teflon-lined cap. If the sample extract requires no further cleanup, proceed with solvent exchange to acetone as described in 10.9. If the sample requires cleanup, proceed to Section 11.
- 10.9 Add 1 or 2 clean boiling chips to the concentrator tube along with 10 mL of acetone. Attach the two-ball macro Snyder column and prewet the column with about 1 mL of acetone. Adjust the temperature of the water bath to 85-95°C. Concentrate the solvent extract as before to an apparent volume of 0.5 mL and allow it to drain and cool for 10 min. Add a second 10 mL of acetone to the concentrator tube and repeat the concentration procedure a second time. Adjust the final volume of the extract to 1.0 mL with acetone.
- 10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

- 11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 The following Florisil cleanup procedure has been demonstrated to be applicable to the four neutral nitrogen pesticides listed in Table 1.

11.2.1 Slurry 20 g of Florisil in 100 mL of ethyl ether and 400 μ L of reagent water. Transfer the slurry to a chromatographic column (Florisil may be retained with a plug of glass wool). Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Wash the column with 25 mL of petroleum ether. Use a column flow rate of 2 to 2.5 mL/min throughout the wash and elution profiles. Add an additional 50 mL of petroleum ether to the head of the column.

11.2.2 Quantitatively transfer the sample from Section 10.8 to the petroleum ether suspended over the column. Allow the solvent to elute from the column until the Florisil is almost exposed to the air. Elute the column with 50 mL of 50% ethyl ether in petroleum ether. Discard this fraction.

11.2.3 Elute the column with 50 mL of 100% ethyl ether (Fraction 1) and collect in a K-D apparatus. Repeat procedure with 50 mL 6% acetone in ethyl ether (Fraction 2), 50 mL 15% acetone in ethyl ether (Fraction 3), 50 mL 50% acetone in ethyl ether (Fraction 4), and 100 mL 100% acetone (Fraction 5), collecting each in a separate K-D apparatus. The elution patterns for the neutral nitrogen compounds are shown in Table 1. Concentrate each fraction to 1 mL as described in Section 10.6 and 10.7. The fractions may be combined before concentration at

the discretion of the analyst. Solvent exchange fraction 1 to acetone as described in Section 10.9 if the fractions are not combined.

11.2.4 Proceed with gas chromatographic analysis.

12. Gas Chromatography

- 12.1 Table 2 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. Examples of the separations achieved by Column 1 and Column 2 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.
- 12.2 Calibrate the gas chromatographic system daily as described in Section 7.
- 12.3 If the internal standard approach is being used, the analyst must not add the internal standard to the sample extracts until immediately before injection into the instrument. Mix thoroughly.
- 12.4 Inject 1 to 5 μL of the sample extract using the solvent flush technique.⁸ Record the volume injected to the nearest 0.05 μL , and the resulting peak sizes in area or peak height units. An automated system that consistently injects a constant volume of extract may also be used.
- 12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the

standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected in μL .

V_t = Volume of total extract in μL .

V_s = Volume of water extracted in mL.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 as follows:

$$\text{Concentration, } \mu\text{g/L} = \frac{(A_s)(I_s)}{(A_{is})(\text{RF})(V_o)}$$

where:

A_s = Response for the compound to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract
in μg .

V_0 = Volume of water extracted, in liters.

- 13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.
- 13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected compounds must be labeled as suspect.

14. GC-MS Confirmation

- 14.1 It is recommended that GC-MS techniques be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 7 seconds per scan utilizing a 70-V (nominal) electron energy in the electron impact ionization mode. A GC to MS interface constructed of all-glass or glass-lined materials is recommended. When using a fused silica capillary column, the column outlet should be threaded through the interface to within a few mm of the entrance to the source ionization chamber. A computer system should be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program.

- 14.2 Gas chromatographic columns and conditions should be selected for optimum separation and performance. The conditions selected must be compatible with standard GC-MS operating practices. Chromatographic tailing factors of less than 5.0 must be achieved.¹⁰
- 14.3 At the beginning of each day that confirmatory analyses are to be performed, the GC-MS system must be checked to see that all DFTPP performance criteria are achieved.⁹
- 14.4 To confirm an identification of a compound, the background corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. It is recommended that at least 50 nanograms of material be injected into the GC-MS. The criteria below must be met for qualitative confirmation.
- 14.4.1 The molecular ion and all other ions that are present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to plus or minus 10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.
- 14.4.2 The retention time of the compound in the sample must be within 6 seconds of the same compound in the standard solution.

- 14.4.3 Compounds that have very similar mass spectra can be explicitly identified by GC-MS only on the basis of retention time data.
- 14.5 Where available, chemical ionization mass spectra may be employed to aid in the qualitative identification process.
- 14.6 Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These may include the use of alternate packed or capillary GC columns or additional cleanup (Section 11).
15. Method Performance
- 15.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.¹¹ The MDL concentrations listed in Table 2 were obtained using reagent water.¹ Similar results were achieved using representative wastewaters.
- 15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 10 x MDL to 1000 x MDL.
- 15.3 In a single laboratory, Battelle's Columbus Laboratories, using spiked wastewater samples, the average recoveries presented in Table 3 were obtained after Florisil cleanup. Seven replicates of each of two different wastewaters were spiked and analyzed. The standard deviation of the percent recovery is also included in Table 3.¹

TABLE 1. ELUTION CHARACTERISTICS OF THE NEUTRAL NITROGEN COMPOUNDS ON 2% DEACTIVATED FLORISIL

Parameter	Elution in Specified Fraction(a)				
	F1	F2	F3	F4	F5
Fenarimol		X	X		
MGK 264		X	X		
MGK 326				X	X
Pronamide	X				

(a) Elution solvents are 50 mL each of the following:

- F1 = 100% ethyl ether
- F2 = 6% acetone in ethyl ether
- F3 = 15% acetone in ethyl ether
- F4 = 50% acetone in ethyl ether
- F5 = 100% acetone (100 mL)

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TABLE 2. CHROMATOGRAPHIC CONDITIONS AND METHOD
DETECTION LIMITS

Parameter	Retention Time (min)		MDL ($\mu\text{g/L}$)
	Column 1	Column 2	
Pronamide	19.9	22.0	4
MGK 326	21.9	23.8	6
MGK 264	23.0 and 23.5(a)	25.5 and 27.5(a)	2
Fenarimol	30.6	32.2	4

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-2250 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is programmed from 80°C to 300°C at 8°C/min with a 4 min hold at each extreme, injector temperature is 250°C and detector is 300°C. Alkali flame detector at bead voltage of 16 V.

Column 2 conditions: Suplecoport (100/120 mesh) coated with 3% SP-2100 packed in a 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. All other conditions as for Column 1.

(a) Two isomers of MGK 264 are resolved from each other.

TABLE 3. SINGLE LABORATORY ACCURACY AND PRECISION(a)

Parameter	Sample Type(b)	Background, $\mu\text{g/L}$ (c)	Spike Level, $\mu\text{g/L}$	Mean Recovery, %	Standard Deviation, %	Number of Replicates
Fenarimol	1	1.8	20	98	4	7
	2	ND	500	96	4	7
MGK 264	1	ND	20	96	23	7
	2	ND	500	74	4	7
MGK 326	1	ND	20	108	7	7
	2	ND	500	95	4	7
Pronamide	1	ND	20	102	5	7
	2	210	500	86	3	7

(a) Column 1 conditions were used.

(b) 1 = Low level relevant industrial effluent.
2 = High level relevant industrial effluent.

(c) ND = Not detected.

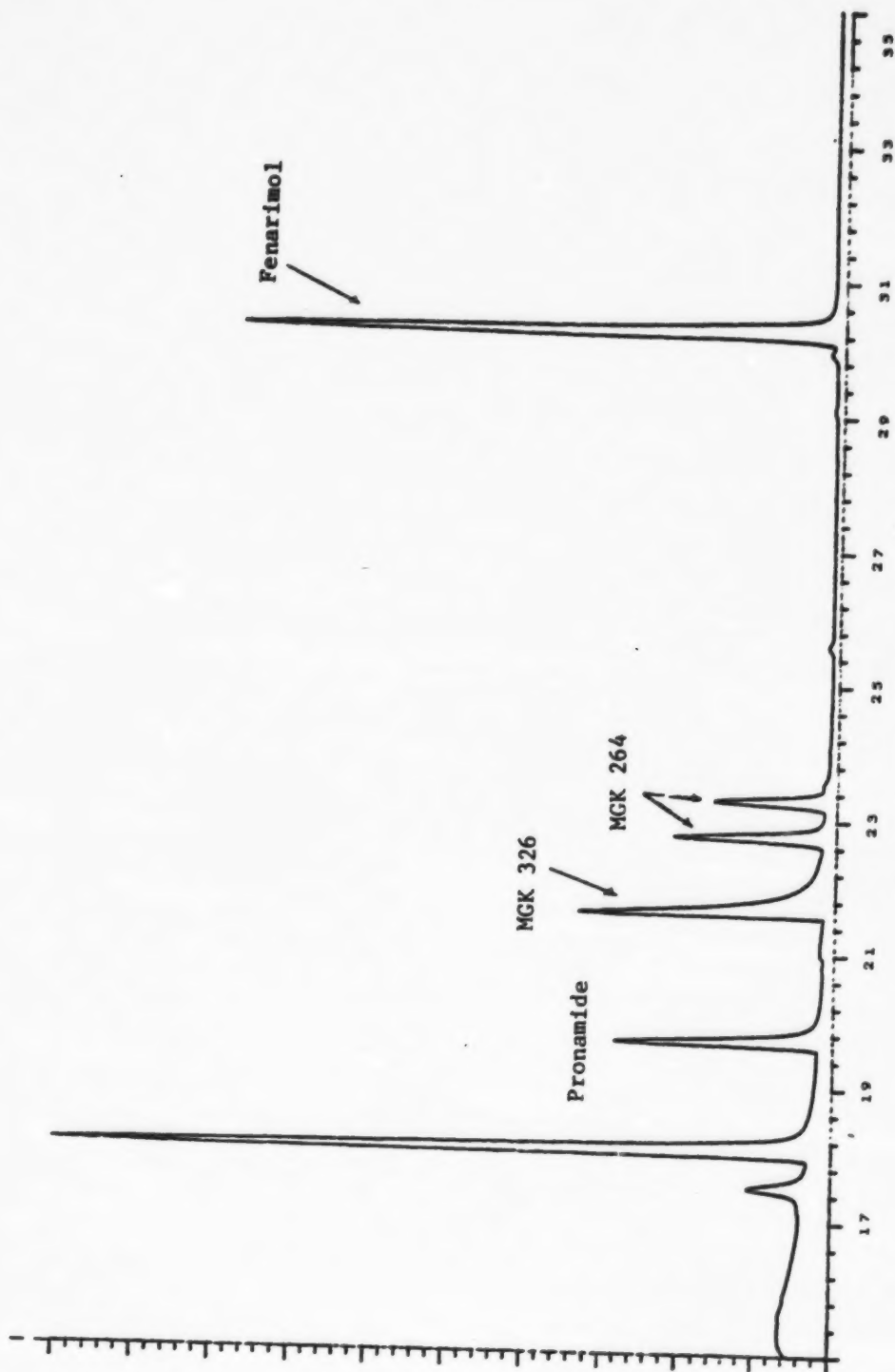


FIGURE 1. GC-AFD CHROMATOGRAM OF 100ng EACH OF THE NEUTRAL NITROGEN COMPOUNDS (Column 1).

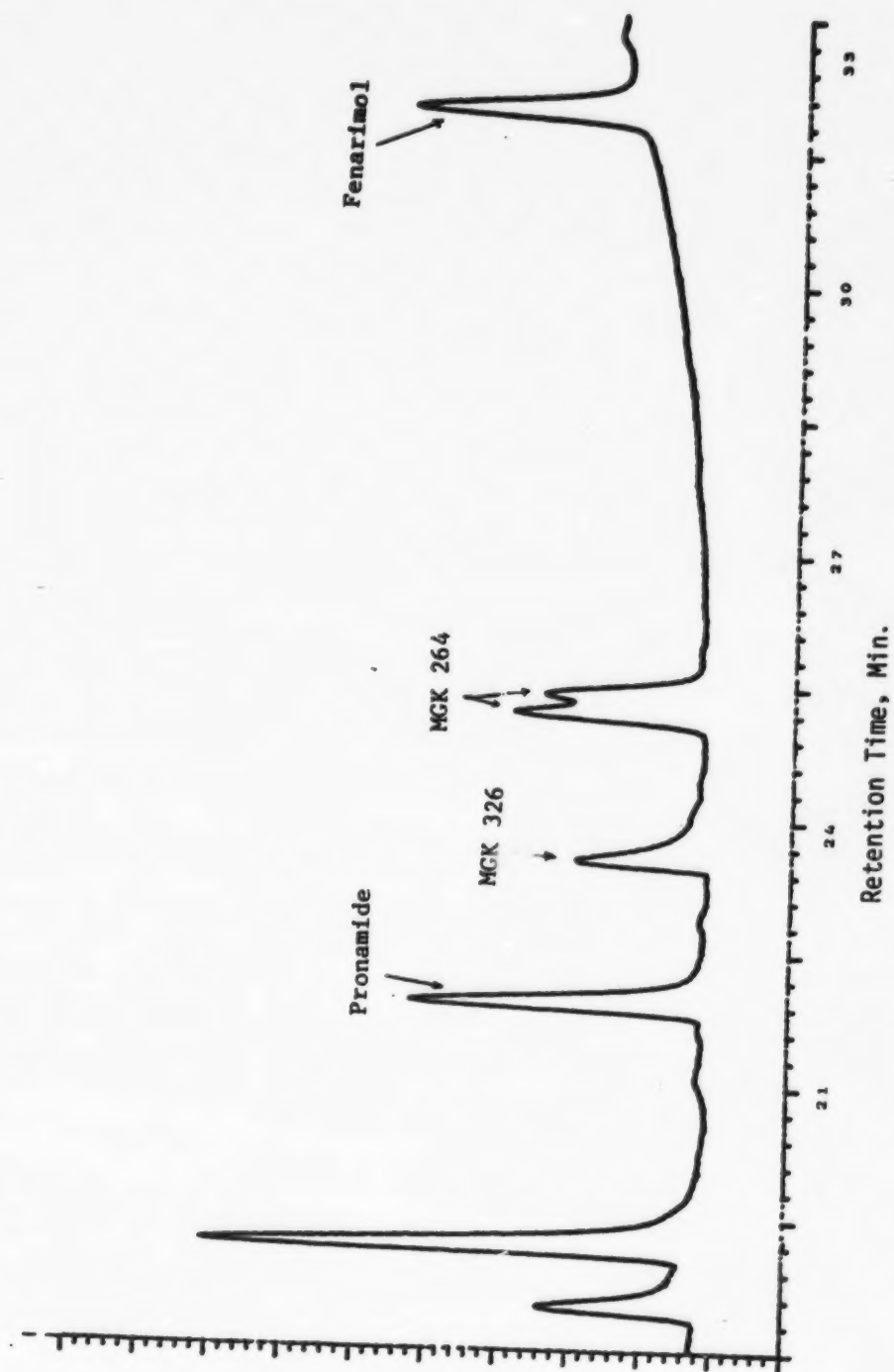


FIGURE 2. GC-AFD CHROMATOGRAM OF 200ng EACH OF THE NEUTRAL NITROGEN COMPOUNDS (COLUMN 2).